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Midlife Body Mass Index and Cerebral Metabolism

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Midlife Body Mass Index and Cerebral Metabolism

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Abstract

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Obesity is a pervasive condition associated with increased risk of dementia, cognitive impairment, and cerebral atrophy in later life. Given that the pathophysiology underlying obesity's impact on the central nervous system is poorly understood, the current study examined the association between body mass index (BMI) and five cerebral metabolites of neurobiological significance: N-acetyl-aspartate (NAA), a marker of neuronal viability; choline-containing compounds, free choline, phosphocholine and glycerophosphocholine (Cho), markers of membrane breakdown and turn over; creatine (Cr), a marker of energy metabolism; myo-inositol (mI), an organic osmolyte and substrate for the synthesis of the secondary messenger, inositol triphosphate; and glutamate (Glu), a marker of excitatory neurotransmission and synaptic integrity. Fifty-five participants, aged 40-60 years, underwent neuropsychological testing, health screen and proton magnetic resonance spectroscopy (^{1}H MRS) of the occipitoparietal grey matter. Concentrations of NAA, Cho, mI, and Glu were calculated as a ratio over Cr and examined in relation to BMI using multivariate multiple regression. Higher BMI was associated with elevations in mI/Cr ($F(5,47)=3.583$, $p=0.008$, ß=0.387, $p=0.006$), independent of age, sex, fasting glucose levels, and systolic blood pressure. The current

study found that higher BMI is related to increased concentrations of the organic osmoltye and glial marker myo-inositol, potentially implicating plasma hypertonicity and neuroinflammation as mechanisms underlying obesity-related brain dysfunction. With validation and absolute quantification, studies of neurometabolites may improve identification of the pathological mechanisms underlying obesity's consequences on cognition.

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1. BACKGROUND AND SIGNIFICANCE

1.1 Overview

Obesity has been declared a global pandemic based upon its striking prevalence across developed nations (1). Rates of overweight and obese status have increased dramatically over the past few decades with current estimates classifying over two-thirds of Americans as overweight or obese based upon body mass index (BMI) (BMI = weight (kilograms) / height^2 (meters^2)) (2). While obesity is most simplistically considered a condition of excess adipose tissue, its sequelae are diffuse, disrupting multiple physiological processes. Through both direct and indirect pathways, obesity damages the heart, liver, and pancreas, increasing susceptibility to a myriad of chronic diseases such as hypertension, diabetes, and cancer (3). Additionally, elevated BMI has been identified as a risk factor for cognitive decline (4-7), suggesting that the health consequences of obesity extend beyond the periphery. Cognitive impairment, even below threshold of dementia, is a significant determinant of long-term institutionalization and dependency in old age, placing strain on the economy and impairing overall quality of life of the aging population and their caregivers (8). Given the widespread prevalence of obesity and the progressive aging population, there is a pressing need to improve identification of the pathological mechanisms underlying obesity's consequences on cognition.

1.2 BMI and Cognition

High BMI has been documented as both a risk for and a protective factor against cognitive impairment depending on the point of examination. High BMI in old age is associated with better cognitive outcomes, while high BMI at midlife increases

vulnerability of future decline (9). In elderly adults, involuntary weight loss precedes the onset of dementia and accelerates with proximity to diagnosis (11). Additionally, a study of 900 elderly Catholic clergymen noted a linear trend between lower body mass and dementia risk with each one-unit decrease in BMI corresponding to a 5% increase in the risk of Alzheimer's disease (12). Yet, studies of elderly adults provide an incomplete picture of the association between obesity and dementia across the lifespan. Body composition changes in old age and co-morbidities that lower body weight, such as respiratory disease and cancer, become increasingly common. Therefore single time-point examination in the elderly may provide a poor estimate of lifetime exposure.

Prospective epidemiological studies that have examined obesity and cognitive outcomes with a lifespan perspective, demonstrate that midlife obesity significantly increases the risk of dementia in old age. A study of community-dwelling middle-aged adults (ages 40-45 years) found that obese BMI was associated with a 3-fold increase in risk for Alzheimer's disease and a 5-fold increase in risk for vascular dementia two decades later (13). Even with adjustment for diabetes and cardiovascular co-morbidities, this relationship remained intact. Similarly, the Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study found that midlife obesity was associated with a 2-fold increased risk for dementia. Co-morbid high cholesterol and high blood pressure had an additive effect with obesity, resulting in a 6-fold increased risk for dementia in old age (14).

Even in the absence of dementia, midlife obesity has negative consequences on cognitive functioning in later life. The Whitehall II Cohort study found that obese BMI

during middle-age was associated with poorer cognitive performance in the domains of memory and executive function in later life (15). Other studies have demonstrated that higher BMI at midlife is associated with sharper cognitive decline across the lifespan (6,16).

Overall, the literature indicates that obesity has a non-linear relationship with cognitive outcomes across the lifespan. In the elderly, higher BMI decreases the risk of dementia (10,11), whereas in younger populations the opposite relationship has been found (14,13). In particular, obese midlife BMI appears to significantly increase the likelihood of dementia and cognitive deficits in old age (6,13-16).

1.3 BMI and Brain Structure

Obesity's impact on cerebral health has been validated by findings of structural brain alterations in human and animal studies. Rats with diet-induced obesity demonstrate reduced hippocampal dendritic spine density (17) and have lower concentrations of synaptic marker proteins such as synapsin and GAP-43 (18). Similarly, in humans, obesity has been associated with hippocampal abnormalities. Greater visceral adiposity, as measured by waist-to-hip ratios, has been correlated with smaller hippocampal size independent of other cardiovascular risk factors (19). A longitudinal study found that obese BMI predicted smaller hippocampal volume 18-years later (20). Given the critical role of the hippocampus in learning and memory (21), these obesity-related structural alternations may translate into poorer cognitive performance. Evidence for this hypothesis has been provided by animal models, which have demonstrated that obesity is associated with reduced long-term potentiation in at the Schaffer collaterals – CA1

synapses (17). In humans, reduced hippocampal volume is an independent risk factor for dementia (22).

Obesity has also been associated with reduced whole brain volume independent of age and other cardiovascular risk factors (23,24). In particular, grey matter reductions have been observed in the orbitofrontal cortex, medial frontal lobes, inferior parietal cortex, lateral occipitipal lobe, cerebellum, and parahippocampus (25). Alterations in white matter have also been demonstrated in obesity. In comparison to wild-type mice, genetically obese ob/ob mice have significantly lower myelin concentrations and alterations in the fatty composition of myelin (26). In humans, obese BMI is associated with larger white matter volumes throughout the frontal, parietal, and occipital lobes (25) with some evidence of reversal with weight loss (27). Overall, the results from these studies suggest that obesity is associated with reduced whole brain and grey matter volumes and increased white matter volumes. Many of the regions displaying structural abnormalities are critical to higher cognitive processes such as attention, planning, and mental manipulation of information (28). Therefore, obesity-related atrophy may translate to poorer cognitive outcomes and increase vulnerability to cognitive decline.

1.4 Proton Magnetic Resonance Spectroscopy (^{1}H MRS)

Proton magnetic resonance spectroscopy (^{1}H MRS) is a non-invasive imaging technique that could potentially inform on the neurochemical alterations associated with obesity. ^{1}H MRS capitalizes on the unique precession properties of atomic nuclei to measure concentrations of cerebral metabolites that reflect cellular structure, membrane integrity, and energetic balance. In brief, ^{1}H MRS relies on the principle that protons,

neutrons, and electrons spin about their axis, creating a quantifiable angular momentum. Additionally, the mass and the positive charge of protons generate a small magnetic field referred to as the magnetic moment. The ratio between the angular and magnetic moments yields a gyromagnetic ratio unique to a particular nucleus. When placed in a magnetic field, the protons will precess at the frequency governed by the Larmor Equation; the strength of the magnetic field multiplied by the gyromagnetic ratio. A precise radio frequency pulse can then be generated to excite the nuclei. The difference between frequency of the emitted signal and the frequency of a reference signal divided by the reference signal yields the chemical shift, expressed in parts per million. The chemical shift is composed of a spread of peaks, each corresponding to different chemicals. The chemicals can be identified by their positions along the frequency axis due to their unique precession frequencies and their concentrations can be derived from the area under their peaks on the chemical shift (29).

When ^{1}H MRS is applied to the brain, several chemicals of neurobiological importance can be identified:

- N-aceytl-aspartate (NAA) is expressed exclusively by neurons and oligiodendrocyte-type-2 astrocyte progenitor cells (30) and is regarded as a marker of neuronal viability (29). Reductions in NAA occur in disease states that result in neuronal loss such as Alzheimer's disease (31), Parkinson's disease (32), and stroke (33). NAA declines that correspond with injury severity and treatment outcomes have been observed after traumatic brain injury (34).

- Choline-containing compounds (free choline, phosphocholine and glycerophosphocholine, Cho), are mainly derived from soluble membrane phospholipids (35). Elevations in Cho concentrations are believed to reflect increased membrane breakdown, myelination, and inflammation (35). Increases in Cho have been observed in patients with malignant tumors (36), multiple sclerosis (31), and HIV infection (37).
- Creatine and phosphocreatine form the CR peak. Due to their role in producing adenosine triphosphate (ATP) from adenosine diphosphate (ADP), their concentrations are thought to reflect energy use (38). Cr concentrations have generally been found stable across the lifespan except in conditions of altered cerebral metabolism such as brain tumors (39). For this reasons, Cr is often used as an internal reference for the quantification of other cerebral metabolite concentrations (29).
- Myo-inositol (mI) is a simple sugar alcohol, which acts as an organic osmolyte and is a precursor for the second messanger inositol tri-phospate (40). Robust increases in mI have been observed in states of chronic hypernatremia (41). Elevations in mI have also been reported in conditions associated with cognitive impairment such as Down's syndrome (42) and Alzheimer's disease (43).
- Glutamate (Glu) is the most abundant excitatory neurotransmitter in the brain (44). Large increases in Glu have been found in states of enhanced cerebral excitation such epilepsy (45) and concussive brain injury (46).

^{1}H MRS has revealed neurometabolite alterations in numerous disorders that correspond to the degree of cognitive impairment and future cognitive outcomes (34,43) Neurometabolite concentrations have also been shown to correlate with cognitive performance in healthy adults (47), implicating their relevance to general cognition. Moreover, ^{1}H MRS has the capability to detect neurochemical alterations in brain tissue prior to the development of inflammatory (31) and cerebrovascular lesions (48), suggesting that it may have greater sensitivity than conventional MRI. Given these parameters, ^{1}H MRS may be particularly adept at identifying subtle cerebral alterations in obese middle-aged adults that are otherwise healthy.

1.5 General Summary

Midlife obesity is an important risk factor for dementia, cognitive decline, and cerebral atrophy in old age (20,4). Given that almost two out of three adults in the United States are classified as either overweight or obese (2) and the progressive aging of the US population, the link between obesity and dementia can easily translate into dire public health consequences. It is therefore crucial to understand the extent to which obesity affects the brain as well as the underlying mechanisms so that measures to prevent cognitive decline can be implemented. Current progress on these preventive efforts has been limited by the low sensitivity of traditional paper-and-pen cognitive tests. Ideally, the impact of obesity on cognition should be assessed before significant irreversible cognitive loss and cerebral atrophy has occurred. In this regard, ^{1}H-MRS may be an ideal methodology because it provides an *in vivo* assessment of cerebral metabolite concentrations, potentially revealing pathogenic mechanisms (49). The overall aim of the

current study was to explore the association between BMI and markers of neuronal viability and metabolism in cognitively intact middle-aged adults using ^{1}H MRS.

2. SUMMARY OF SPECIFIC AIM

In elderly adults, high BMI has been identified a protective factor against dementia (9). However, epidemiological studies with longer follow-up periods have found that high BMI at midlife increases the risk of cognitive impairment (4,14) and correlates with the degree of cerebral atrophy in old age (19,20). The current study proposes to provide a real-time assessment of the association between midlife BMI and concentrations of cerebral metabolites with neurobiological significance using ^{1}H MRS. Concentrations of five metabolites (NAA,Cho, Cr, mI, and Glu) were analyzed within the occipitoparietal grey matter. The region was selected because neurometabolite changes that correlate with the degree of cognitive impairment have been reliability detected in populations with cognitive disorders (43). It was hypothesized that higher BMI, as a risk factor of cognitive decline and structural brain alterations, would be associated with lower concentrations of NAA and higher concentrations of mI. NAA is a marker of neuronal viability (29) and lower levels have been identified in cognitive disorders with neuronal loss (32,34,43) and in healthy adults with poorer cognitive performance (47). mI is an organic osmolyte (50) which demonstrates elevations during the premorbid stages of cognitive disorders (43). NAA and mI were predicted to correlate with higher BMI due their sensitivity in detecting early cognitive vulnerability.

3. MATERIALS AND METHODS

3.1 Participants

Right-handed adults between the ages of 40 and 60 years were recruited through flyers and newspaper advertisements. Individuals with a history of coronary artery disease, angina pectoris, myocardial infarctions, heart failure, and cardiac surgery were excluded. Additional exclusion criteria included history of neurological disease (e.g., stroke, Parkinson's disease, clinically significant traumatic brain injury), major psychiatric illness (e.g. schizophrenia, bipolar disorder), substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse), smoking (within the last two years), and MRI contraindications. Fifty-five participants completed the initial screen and were enrolled in the study after providing written consent. Two participants were excluded from analyses were excluded from the analyses due to poor quality MRS data (Cramer-Rao Lower Bounds for NAA/Cr, mI/Cr, Cho/Cr or Glu/Cr >12). Participant characteristics by group are presented in Table 1.

3.2 Procedures

The study was approved by the Institutional Review Board of the University of Texas at Austin and all volunteers provided written informed consent before enrollment. Participants were required to complete a medical history interview in which medical conditions and treatments were coded as either present or absent based on participants' self-report. Participants then underwent a full neuropsychological evaluation, brain imaging, and a general health assessment, including a fasting blood draw for lipid and

glucose assay. Visits were conducted on separate days and participants completed the study within one month.

3.2.1 Neuropsychological Assessment

All participants completed a two-hour assessment battery including standard clinical neuropsychological instruments with established reliability and validity (51). All tests were administered and scoring using standard administration and scoring criteria.The battery included the following tasks:

- Mini Mental Status Exam (MMSE) (52): a 30-item test of general mental status including arithmetic, memory, and orientation. Scores below 24 indicate possible dementia.
- Wechsler Abbreviated Scale of Intelligence (WASI) (53): a test of general intelligence. The two subtest format consists of Vocabulary and Matrix Reasoning. Vocabulary is a measure of word knowledge with a maximum of 80 possible points. Matrix Reasoning is a measure on non-verbal reasoning by analogy and has maximum of 35 (12-44 years) or 32 (45-79 years) total points. The two subtests can be used to calculate a full-scale IQ composite score with a mean of 100 and a standard deviation of 15.
- Category Fluency for Animals (54): a measure of semantic fluency. Participants must name as many animals as they can in 1-minute.
- Rey Complex Figure Test (RCF)(55): a measure of visiospatial constructional ability and visiospatial memory. Participants must copy a complex figure, which they must recall and reconstruct immediately after stimulus presentation and after

a 45-minute delay. Following the delay, participants also complete a recognition test. The copy and free recalls are scored by giving credit to figure components that are correctly drawn and accurately place with a maximum of 36 total points. Partial credit is awarded for items that are incorrectly drawn but recognizable. For the recognition subtest, 1-point is awarded to each component that is correctly identified as being included or not included in the original figure with a maximum of 24 total points.

- California Verbal Learning Test-II (CVLT-II) (56): a measure of verbal learning and memory. A 16-item word list is presented five times and participants must recall as many words as they can after each presentation. Following the presentation of a 16-word distracter list, participants are asked to recall the original list with and without semantic cuing. After a 20-mintute delay, participants recall the original list with and without semantic cuing and complete a yes/no item recognition test. Free recall trials have a maximum of 16 points with 1-point awarded for each correctly recalled item from the list. A recognition discriminability index (d') is calculated from the yes/no item recognition section. The calculation of d' is based on the number of hits, number of possible total hits, number of false-positives, and number of total possible false-possible, yielding a maximum score of 4.0.
- Wechsler Adult Intelligence Scale-III (WAIS-III) Digit Span Subtest (57): a measure of apprehension span and working memory. On Digits Forward, participants must repeat sequences of verbally presented numbers. On Digits

Backwards, participants must recall the numbers in reverse sequence. 1-point is awarded for each correctly sequenced item on Digits Forward and Backwards with their composite sums creating a Digits Total Score.

- Controlled Oral Word Association Test (COWAT) (58): a measure of verbal fluency. Participants must name as many words as they can beginning with a specified letter within a 1-minute time frame.
- Grooved Peg Board, Dominant hand (59): a measure of fine motor coordination. Participants must rotate key-shaped pegs to fill the 25 holes on the board as quickly as possible. Time to completion is recorded for both the dominant and non-dominant hands.
- Trail Making Test A & B (60): a measure of visual attention and processing speed. For part A, participants draw a line connecting 25 randomly dispersed numbers in numerical order. On part B, participants must alternate between connecting randomly dispersed numbers and letters in sequence (1-A-2-B-3-C). Time to completion is recorded for both parts.
- Beck Depression Inventory-II (BDI-II) (61): a 21-item self-report questionnaire assessing depression. The maximum scores is 63, with scores greater than 14 suggesting clinically significant depression symptoms
- Spielberger Trait Anxiety Inventory (STAI-T) (62): a 20-item self-report questionnaire on trait anxiety with higher scores representing greater anxiety.

3.2.2 Imaging Protocols and MRS Data Processing

MRS data for each participant were acquired in a single session on a 3T GE Signa Excite MRI scanner equipped with a standard head coil. Imaging included single voxel proton MRS performed using the GE pulse sequence PROBE-P, an automated point resolved spectroscopy (PRESS) sequence with chemical shift selected (CHESS) water suppression. Each spectroscopic voxel was prescribed from 3D high-resolution Spoiled Gradient Echo (SPGR) sagittal images (256 x 256 matrix, FOV = 24 x 24 cm^2, 1 mm slice thickness, 0 gap) of the entire brain. ^{1}H-MRS parameters were as follows: echo time/ repetition time (TE/TR) = 35/3000 ms, 128 excitations, 5000 Hz spectral width, volume ~6 cm^3 from the occipitoparietal gray matter including posterior cingulate gyrus (Fig. 1). Neurometabolite concentrations in this region have consistently been found to correlate with cognitive function (34,43). Commercially available software, LCModel, was used to quantify and separate the metabolite resonances from the macromolecule background (63) (Fig. 2). In line with standard clinical protocols, the concentrations of NAA, Cho, mI, and Glu were reported as ratios relative to creatine (Cr) (43).

3.2.3 General Health Assessment

Participants abstained from caffeine and fasted for at least four hours prior to the assessment. Body weight in kilograms and height in centimeters were measured on a beam-balance scale for the subsequent calculations of BMI. BMI was calculated by dividing weight in kilograms by height in meters squared. Following 15 minutes of rest, participants sat upright while brachial blood pressure was measured using a semi-automated device. Approximately 3 mL of fasting blood was collected from the

antecubital vein by venipucture. The concentrations of glucose, triglycerides, LDL-cholesterol, and HDL-cholesterol were measured using standard enzymatic technique.

3.3 Statistical Analyses

Neuropsychological measures were grouped into one of five cognitive domains: 1) *global cognitive functioning*, 2) *language functions*, 3) *visual-spatial abilities*, 4) *memory functions*, and 5) *attention-executive functions*. The following test scores were included in each domain and raw total scores were utilized unless otherwise stated: 1) *global*: MMSE (52) and WASI Full Scale IQ (53); 2) *language*: WASI Vocabulary Subtest (53) and Category Fluency for Animals (54); 3) *visual-spatial*: RCF copy (55) and WASI Matrix Reasoning Subtest (53); 4) *memory*: CVLT-II immediate recall, delayed recall, and recognition discrimination (56), RCF immediate recall, delayed recall, and recognition discrimination (55); 5) *attention-executive- functions*: Trail making A and B time to completion (60), COWAT (58), WAIS-III Digit Span Subtest (57), and Grooved Pegboard-Dominant Hand time to completion (59). Participants' raw test scores were converted to z-scores using the study sample mean and standard deviation. Timed test scores were multiplied by -1 so that higher scores indicate better performance. Five composite cognitive domain z-scores were calculated for each participant by averaging the z-scores of all tests within that domain.

Descriptive statistics were calculated for demographics, medical variables, and raw cognitive test scores. Cognitive domain score scores were assessed in relation to BMI and ^{1}H MRS markers (NAA/Cr, Glu/Cr, mI/Cr and Cho/Cr) using linear regression controlling for age and years of education. The association between BMI and the ^{1}H

MRS markers was analyzed using a single multivariate multiple linear regression model with all MRS parameters entered in at once, controlling for age, sex, fasting glucose levels, and systolic blood pressure. The above analysis was repeated using global cognition, anti-hypertensive medications, and hypoglycemic medications as additional covariates. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL). A two-tailed alpha level of 0.05 was used as the criterion for statistical significance.

4. RESULTS

4.1 Descriptive Statistics

Means and standard deviations of the demographic and medical variables of the sample are reported in Table 1. Table 2 displays the mean raw cognitive test scores and their standard deviations. Descriptive statistical analyses revealed a cognitively normal, ethnically diverse, middle-aged sample, well representative of the population of the state of Texas based on year 2000 US census data for the state. Mean BMI was 29.4 kg/m^2 with a range from 19.0 to 42.8 kg/m^2. Using the World Health Organization's BMI categorization, 16 participants (28.1%) had a normal BMI (18.5–24.9 kg/m^2), 15 participants (26.3%) had an overweight BMI (25.0–29.9 kg/m^2), and twenty-six participants (45.6%) had an obese BMI ($\geq$30 kg/m^2). Eleven participants were currently being treated with anti-hypertensive medications, three with lipid lowering agents, four with hypoglycemics, three with biphosphonates, one with thyroid replacement therapy, and one with antidepressant medications.

4.2 BMI in Relation to Cognitive Test Performance

BMI was not significantly associated with performance on measures of language ($F(3,51)=5.090$, $p=0.004$, ß=-0.131, $p=0.309$), visual-spatial abilities ($F(3,51)=0.375$, $p=0.772$, ß=-0.096, $p=0.512$), memory ($F(3,51)=1.318$, $p=0.280$, ß=0.064, $p=0.651$), or attention-executive functions ($F(3,51)=0.586$, $p=0.627$, ß=-0.150, $p=0.297$) independent of age and years of education. However, there was a trend towards higher BMI relating to lower global cognitive function ($F(3,51)=3.776$, $p=0.016$, ß=-0.287, $p=0.052$).

4.3 Cerebral Metabolism in Relation to Cognitive Test Performance

Consistent with the literature on mild cognitive impairment (43), higher levels of mI/Cr were associated with lower performance in the memory domain independent of age and years of education ($F(3,51)=2.077$, $p=0.040$, $ß=-0.290$, $p=0.030$). No significant relationship was found between NAA/Cr, Glu/Cr, and Cho/Cr and any domain of cognitive functioning independent of age and years of education.

4.4 Cerebral Metabolism in Relation to BMI

The fully adjusted multivariate regression model successfully predicted the level of mI/Cr in occipitoparietal grey matter ($F(5,47)=3.583$, $p=0.008$), but not NAA/Cr ($F(5,47)=1.273$, $p=0.291$), Cho/Cr ($F(5,47)=1.393$, $p=0.244$) or Glu/Cr ($F(5,47)=1.526$, $p=0.200$). Higher body mass index was significantly associated with higher mI/Cr ($ß=0.387$, $p=0.006$), independent of age, sex, fasting glucose levels, and systolic blood pressure (Fig. 3). This relationship remained unchanged when global cognition function was included in the model ($F(6,45)=3.099$, $p=0.013$, $ß=0.389$, $p=0.010$). Additionally, controlling for the use of anti-hypertensive ($F(6,45)=2.935$, $p=0.017$, $ß=0.376$, $p=0.012$) or hypoglycemic medications ($F(6,45)=2.889$, $p=0.018$, $ß=0.394$, $p=0.006$) did not alter the significance of the findings.

5. DISCUSSION

5.1 Summary of Findings

The main finding from the study was that higher BMI was associated with elevations of mI/Cr in the occipitoparietal grey matter independent of age, sex, systolic blood pressure, and fasting glucose levels. Elevations in mI were predicted based on similar findings in other cognitively vulnerable population (42,43). However, the predicted relationship between higher BMI and lower NAA was not observed. Reductions in NAA frequently co-occur with elevations in mI in cognitively vulnerable populations (43,64). However, reductions in NAA and elevations in mI do not necessarily correlate with one another (64), suggesting that they reflect different pathological processes. NAA is a marker of neuronal viability and reductions have typically been observed in disorders with cerebral atrophy (29). In contrast, mI is an organic osmolyte and proposed maker of gliosis (40). The findings suggest that osmotic regulation and/or glial cell alterations may precede the neuronal loss associated with higher BMI.

5.2 Myo-inositol and Cognition

Elevations in mI/Cr have been noted in numerous populations with cognitive deficits such as those with mild cognitive impairment (65) and Alzheimer's disease (43). In these populations, mI/Cr has demonstrated significant associations with the degree of cognitive decline (65,43). Elevations in mI/Cr have also been noted in adults with Down syndrome (42) and individuals at genetic risk for Alzheimer's disease (66) before the onset of dementia, suggesting that alterations in mI/Cr emerge early in the pathogenic process to dementia. In the current study, higher levels of mI/Cr were related to lower

scores on the memory domain despite overall normal cognitive performance. These results suggest that individuals with higher BMI may be demonstrating alterations in neurochemistry associated with increased cognitive vulnerability. Further work will be necessary to determine the utility of these findings for future cognitive trajectories.

5.3 Myo-inositol's Role in the Central Nervous System

Myo-inositol is a simple sugar alcohol with high cerebral concentrations (50). The neurometabolite can be produced *de novo* from glucose within the brain or it can be transported from the plasma (67). Due to its high concentrations within glial cells, myo-inositol is a proposed marker of gliosis (43,68). Additionally, myo-inositol is an organic osmolyte and a precursor for membrane phospholipids and for the second messenger inositol tri-phosphate (50). High levels of myo-inositol have been reported in patients with diabetes (69-71) and hypertension (72), conditions closely related to obesity. In the current study, BMI was associated with elevations in mI/Cr even after controlling for systolic blood pressure, fasting glucose levels, anti-hypertensive medications, and hypoglycemic agents, suggesting that high BMI uniquely accounted for some of the variance in mI/Cr values in the occipitoparietal grey matter.

5.4 Myo-inositol, Obesity, and Osmotic Regulation

Myo-inositol's role as organic osmolyte may explain its association with BMI. Neural and non-neural cells within the brain experience constant fluctuations in the concentrations of electrolytes transported across the plasma membrane. This must be closely regulated to prevent large alterations in the water diffusion, which could lead to cell death or damage by shrinkage or swelling. Acute changes in electrolyte

concentrations are regulated by ions but during chronic conditions organic osmolytes resume this function (73). Chronic hypernatremia induces robust increases in myo-inositol concentrations in the brain that is governed by sodium dependent myo-inositol cotransport (41,74,73). The upregulation of the sodium dependent myo-inositol cotransport and subsequent increase in brain myo-inostiol is extremely sensitive to osmotic stress, so even small, persistent changes in plasma tonicity may increase myo-inositol concentrations in the brain (73).

Interestingly, overweight and obese individuals have higher extracellular relative to intracellular body fluid (ECF/ICF) that is presumed to be due to higher plasma tonicity (75). Glucose dysregulation is a well-documented cause and consequence of both obesity (76) and plasma hypertonicity (77). A prior study found that obese individuals were 50% more likely to have plasma hypertonicity than lean controls even after controlling for plasma glucose (75). Plasma hypertonicity in the obese individuals was strongly associated with higher plasma sodium concentrations in addition to elevated glucose levels. Evidence also suggests that obese individuals may have altered ECF/ICF in the brain. Diffusion weighted imaging demonstrated that obese individuals have higher apparent diffusion coefficient (ADC) values in numerous regions throughout the brain in comparison to lean controls (78). ADC values represent the diffusion of water molecules within a tissue and vasogenic edema, a condition associated with an increase in extracellular water distribution, is related to higher ADC values (79). Similarly in obese individuals, higher ADC values may represent an increased ECF secondary to plasma hypertonicity (78). In such a state, cerebral concentrations of myo-inositol would be

upregulated to maintain osmotic balance. Thus, plasma hypertonicity may be a mechanism linking higher BMI to elevations in cerebral myo-inositol.

5.5 Myo-inositol, Obesity, and Inflammation

Another condition that may explain the association between myo-inositol and BMI is gliosis subsequent to inflammation. Insult or injury in the brain induces astrocyte proliferation and the presence of activated microglia, which release inflammatory cytokines and oxidative radicals (80). Myo-inositol resides primarily in glial cells so inflammatory-induced gliosis may increase its cerebral concentrations (43,68). Accordingly, elevations in myo-inositol have been detected in several conditions associated with neuroinflammation such as multiple sclerosis (31), acquired immunodeficiency syndrome (81), and Alzheimer's disease (43).

Inflammation is also a well-established symptom of obesity. Adipose tissue secretes numerous cytokines, some which are capable of crossing the blood brain barrier and initiating a local proinflammatory response (82). In rodents, high fat diets have been shown to increase astrocyte proliferation, activated microglia, and brain levels of proinflammatory cytokines (83). Furthermore, leptin, a hormone secreted in proportion to adipose tissue, can cross the blood brain barrier and alter inflammatory signaling in microglia (84). Obesity-related inflammation may therefore be a potential mechanism responsible for elevated myo-inositol concentrations.

5.6 Comparison with Previous Findings

The current study found a strong association between myo-inositol and BMI. Notably, two published ^{1}H MRS studies examining BMI did not find significant effects

for myo-inositol levels (85,86). Examining middle-aged adults, Gadzinksi et al. (2008) (85) reported that greater BMI was associated with lower NAA/Cr levels in the frontal, parietal, and temporal white matter, lower NAA/Cr in the frontal grey matter, and lower Cho/Cr in the frontal white mater. In a similar study of older adults, Gazdzinski et al. (2010) (86) found that BMI was related with decreased NAA/Cr and Glu/Cr in the anterior cingulate. The differences in findings may be due to regional tissue variation in neurometabolite concentrations. The current study examined a voxel within the occipitoparietal lobe. When the prior studies examined posterior brain regions, they also found no association between NAA/Cr and BMI. Nonetheless, elevations in mI/Cr concentrations are a unique finding in the current study. This discrepancy could be explained methodological variance. The study conducted by Gazdzinski et al. (2008) (85) acquired data from a 1.5-Telsa scanner with an echo time/ repetition time (TE/TR) = 25/1800 ms. In Gazdzinski et al. (2010) (86) a 4-Telsa scanner with a TE/TR = 15/2000 was utilized. The current study was conducted on a 3-Telsa scanner with a TE/TR = 35/3000 ms. Differences in magnetic field strength and TE/TR parameters can alter quantifiable precision due to changes in the signal-to-noise ratio and chemical shift dispersion (87). The variation in findings between the current study and previous ones may also be explained by differences in the participant sample. In the current study, a relatively large proportion of participants were obese (45.6%) in comparison to the sparse representation of obesity in the other two studies, 10% (85) and 0% (86) respectively. It is possible that perturbations of mI/Cr do not emerge until BMI becomes pathologically elevated.

5.7 Study Limitations

While contemplating the results of the study, it is important to consider its strengths and limitations. The primary strength of the current study was its detailed characterization of the study participants in terms of medical history and cognitive function. All participants underwent a structured medical history interview to help avoid a potential under-reporting bias in response to self-report forms. The detailed cognitive assessment provided a thorough assessment of participants' cognitive functioning across multiple domains including global cognitive functioning, language functions, visual-spatial abilities, memory, and attention-executive-psychomotor functions. The study also included objective assessment of physiological indices such as fasting glucose levels and systolic blood pressure. This enabled us to control for common co-morbidities associated with obesity and cerebral alterations in order to determine BMI's independent impact on cerebral neurochemistry. A limitation of the study was that the overall sample size was relatively small, so the present findings must be considered preliminary. Also, the occipitoparietal region that was sampled contains mostly grey matter but does inherently include some white matter. Neurometabolite concentrations have been noted to vary between grey and white matter tissue (35). Changes in grey/white matter composition have been reported in association with BMI (25), so tissue segmentation may be a useful correction. The current study performed spectroscopy on a single region of interest. Inclusion of multiple voxels would provide better characterization of BMI-related alterations across the cerebral cortex. Finally, the cross-sectional nature of the study is a limitation. It is impossible to determine if alterations in mI are pre-existing or develop as

a result of higher BMI. It is also unclear as to whether mI elevations would reverse with successful weight loss. Most importantly, longitudinal studies will be critical in determining whether the observed alterations in neurochemical concentrations are predictive of individual cognitive trajectories.

5.8 Summary

In conclusion, the main finding of the current study was that higher BMI was associated with elevations in mI/Cr concentrations in the occipitoparietal grey matter independent of age, sex, systolic blood pressure, and fasting glucose levels. This finding adds to the accumulating literature demonstrating that obesity has an adverse impact on central nervous system functioning. Several studies have indicated that obesity is a risk factor for dementia and cognitive decline and relates to structural alterations in brain volume. The current study provides evidence that higher midlife BMI is related to increased cerebral mI/Cr ratio, potentially implicating plasma hypertonicity and neuroinflammation as mechanisms underlying obesity-related brain dysfunction. Longitudinal studies will be necessary to determine if the observed neurometabolic alterations are predictive of cognitive vulnerability. With validation and absolute quantification, studies of neurometabolites may help uncover the pathological mechanisms underlying obesity's impact on cognition.

APPENDIX A. TABLES

Table 1. Participant Characteristics

	Mean±SD
Age, y	50.8±6.6
Sex (male/female), n	25:28
Race, n (%)	
Caucasian	21 (39.6%)
Hispanic	24 (45.4%)
African American	4 (7.5%)
Other	4 (7.5%)
Education, y	15.2±2.9
Body mass index, kg/m2	29.6±5.9
Systolic blood pressure, mmHg	124.9±15.7
Diastolic blood pressure, mmHg	75.4±8.8
LDL-Cholesterol, mg/dl	124.3±34.0
HDL-Cholesterol, mg/dl	48.4±16.7
Triglyceride, mg/dl	166.8±97.4
Glucose, mg/dl	104.5±30.7

Table 2. Neuropsychological Test Results

Cognitive Domain	Mean±SD
Global cognition	
Mini Mental Status Exam (MMSE)	28.4±1.3
Weschler Abbreviated Test of Intelligence (WASI)	113.5±11.9
Full Scale IQ	
Language	
WASI - Vocabulary Subtest	63.9±10.0
Category Fluency for Animals	23.4±5.7
Visual-Spatial	
WASI Matrix Reasoning Subtest	25.9±4.3
Rey Complex Figure Test (RCF) - Copy	30.3±3.9
Memory	
California Verbal Learning Test (CVLT)	
Immediate Recall	10.9±3.0
Delayed Recall	11.2±3.2
Recognition (Yes/No)	3.1±0.8
Rey Complex Figure Test (RCF)	

Immediate Recall	16.0±5.4
Delayed Recall	15.5±5.5
Recognition Discrimination	19.2±3.8
Attention-executive function	
Controlled Oral Word Association Test (COWAT)	37.3±10.8
Trail Making Test A, sec	32.0±10.7
Trail Making Test B, sec	80.0±31.0
Weschler Adult Intelligence Scale III (WAIS-III) Digit Span Subtest, total	16.1±4.1
Grooved Pegboard, Dominant Hand, sec	77.2±14.0
Emotional function	
Beck Depression Inventory II (BDI-II)	6.1±6.3
Spielberger Trait Anxiety Inventory	33.9±9.1

APPENDIX B. FIGURES

Figure 1. MRS Voxel Borders on High Resolution Anatomy Indicating Volume of Interest in Occipitoparietal Junction

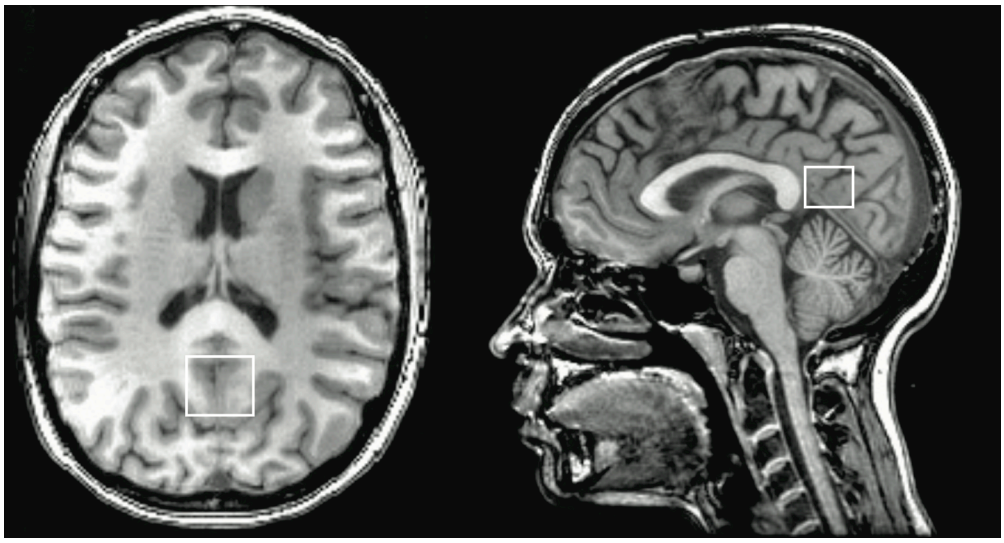


Figure 2. Representative ^{1}H MRS Spectrum

The narrow line width and small residual indicate excellent model fit. NAA = N-acetyl-aspartate; Glu = glutamate; Cr = creatine + phosphocreatine; Cho = choline + phosphocholine; mI = *myo*-inoitol

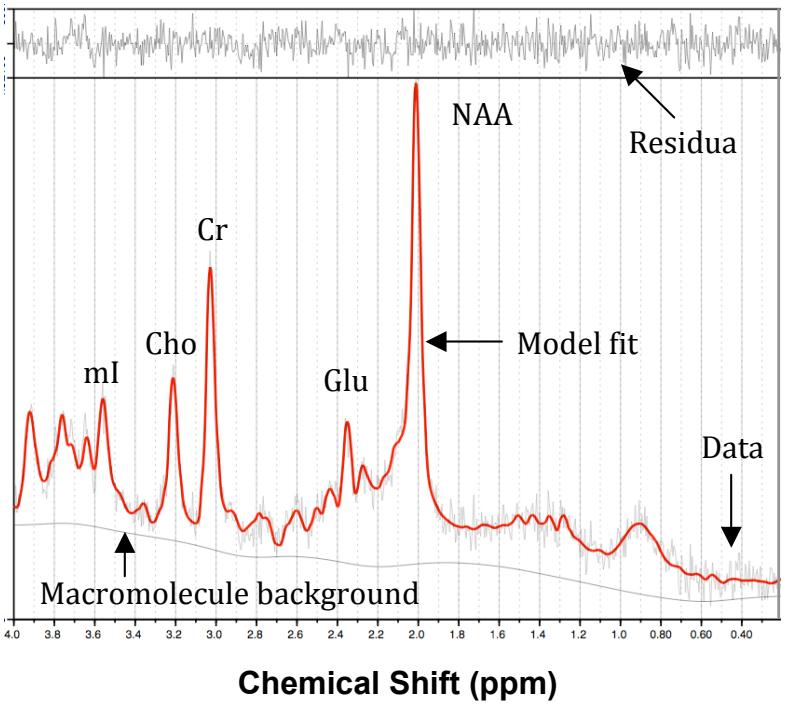
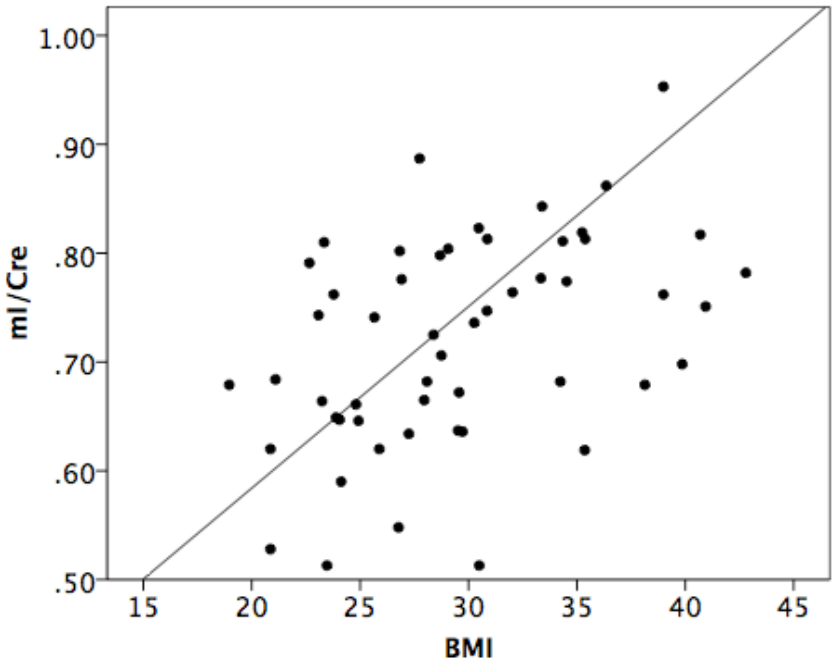


Figure 3. Scatterplot Displaying the Relation between BMI and mI/Cr



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